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FS-SO-2203-1.37
(Problem 2)

FINAL REPORT

NITROGEN UTILIZATION BY THE SOUTHERN PINE BEETLE (SPB)

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INTRODUCTION

The southern pine beetle, *Dendroctonus frontalis* Zimm. (SPB), lives in association with many species of microorganisms. Two species of fungi are obligate symbionts and are carried in a special organ or mycangium (Barras and Perry 1972). Growth and development of the SPB are greatly reduced in the absence of these mycangial fungi (Barras 1973). The reasons for this dependency of the SPB on its symbionts are not known, but the fungi possibly serve a nutritional role.

Colonization of pine bark by mycangial fungi decreases the reducing sugar content of the bark (Barras and Hodges 1969). The SPB-microorganism complex also increases the concentration of protein-bound amino acids and total nitrogen of bark tissue (Hodges et al. 1968). Whether these changes are important to beetle development is not known.

Because the total nitrogen content of pine bark is relatively low, this nutrient might limit the growth of SPB, and the symbiotic microorganisms could function to provide the necessary additional nitrogen to the beetle. This study was done to determine the nitrogen utilization of the SPB by measuring the efficiency of conversion of ingested nitrogen to body tissue. This nutritional index could be used to evaluate whether nitrogen might be the basis for the symbiosis of SPB with its symbiotic fungi.

MATERIALS AND METHODS

In December 1978, a loblolly pine tree was felled, and the lower bole was cut into 7 bolts approximately 60 cm long. Six of the bolts were inoculated with pairs of emergent SPB collected from naturally infested pines. The seventh bolt was used as an uninoculated control. One of the inoculated bolts had no beetle development and was discarded. After 3 weeks the outer bark was carefully removed, and SPB larvae and their frass were collected. Care was taken to collect all the frass from each larva. Frass was suctioned into pre-weighed tubes 6 cm long made from a 1-ml disposable pipette plugged with glass wool and connected to a vacuum line. Frass from 10 larvae was collected in each tube. Frass samples were then freeze-dried and weighed.

Inner bark was collected from around the larval gallery to a distance of 3-5 cm. Uninfested bark was collected from an area of each bolt having no beetle galleries. The bark was freeze-dried and then ground in a Wiley mill with a 40-mesh screen. Total nitrogen was determined by a modification of the method of Umbreit et al. (1972). To digest the samples, at least 10 ml of concentrated sulfuric acid was used per gram of dry sample (Bremner 1965).

The efficiency of conversion of ingested nitrogen (E.C.I.(N)) is given by the following formula (Waldbauer 1968).

$$E.C.I.(N) = \frac{\text{Amount of nitrogen in body}}{\text{Amount of nitrogen in food ingested}} \times 100$$

The amount of bark ingested by each larva could not be determined directly. Therefore, "amount of nitrogen in body + amount of nitrogen in frass" was substituted for "amount of nitrogen in food ingested". This assumes that all the nitrogen ingested is eventually found in the larva's body or frass. That is, nitrogen is not lost as a volatile compound or is not leached out of the frass into the surrounding bark.

RESULTS

Total nitrogen concentration of pine inner bark near SPB galleries was significantly greater than that of uninfested bark (Table 1). Uninfested inner bark had 0.43% nitrogen, compared to 0.69% for infested bark.

The E.C.I. (N) was calculated for SPB larvae. Larvae averaged 1.0 ± 0.05 mg (mean \pm standard error of the mean) in weight and produced a mean of 3.1 ± 0.2 mg of frass. Nitrogen concentration of larvae averaged 53.5 ± 2.1 μ g/mg of dry weight. Frass had a mean nitrogen concentration of 8.9 ± 0.4 μ g/mg of dry weight. E.C.I. (N) averaged $66.7\% \pm 1.7$. This value indicates that 66.7% of the nitrogen ingested by larvae was converted to body tissue.

DISCUSSION

This study was undertaken as part of an analysis of the relationship of the SPB to its symbiotic fungi in terms of nitrogen nutrition. The importance of nitrogen to wood utilizing insects is well established (Bletchly and Farmer 1959, Bletchly and Taylor 1964). Although nitrogen is higher in bark than wood, it could be the nutrient limiting SPB growth.

Total nitrogen was 60.5% higher in bark near SPB galleries than in infested bark. This increased concentration of nitrogen is probably important for beetle development. The E.C.I. (N) by SPB larvae is relatively high compared to some other insects (Waldbauer 1968). For larvae to develop in uninfested bark their utilization of available nitrogen would have to be increased or they would have to consume much more food. The E.C.I. (N) could be increased by better digestibility of nitrogen or by increased conversion of digested nitrogen into body tissue. Better digestibility of nitrogen in uninfested bark might be possible. Uninfested bark has a higher concentration of soluble amino acids than infested bark. Whereas in infested bark more of the amino acids are as protein-bound nitrogen (Hodges et al. 1968). It is possible that free amino acids might be utilized better than protein-bound ones, and it is conceivable that larvae could have better utilization of nitrogen in uninfested bark. But, because larvae utilize a relatively large percentage of ingested nitrogen, they could not increase their efficiency of nitrogen utilization enough to compensate for the 60% increase in nitrogen in infested bark. Assuming that the higher nitrogen concentration near bark beetle galleries is caused by symbiotic microorganisms, then symbionts probably play an obligatory role as nitrogen suppliers to the developing larvae.

The mechanism for the increase in nitrogen by SPB/microbial complex is not fully understood. Nitrogen-fixation has been suggested (Hodges et al. 1968). However, Bridges (1981) found no nitrogen-fixing bacteria associated with SPB larvae. One mechanism for the increase in nitrogen probably involves loss of carbon due to metabolism by microorganisms. Evolution of CO_2 through microbial respiration would result in an increase in nitrogen concentration.

In wood, nitrogen and other nutrients are concentrated at surfaces where drying occurs (Long 1978, King et al. 1974). The increase in nitrogen concentration in wood is more than can be accounted for by carbohydrate loss and is not due to inorganic nitrogen accumulation (Waite and King 1979). During drying of wood soluble nitrogen migrates to the surface from which evaporation occurs where it is deposited in concentrations of up to 5 times the average levels of total nitrogen (King et al. 1974). A similar situation could occur in bark beetle galleries. As the bark tissue dries along the galleries (Webb and Franklin 1979), soluble nitrogen migrates to these areas. What has not been established is whether soluble nitrogen migrates passively by diffusion to the drying surfaces or whether symbiotic microorganisms stimulate an active translocation of nitrogen. It has been suggested that nitrogen redistribution is an active process by the tree in response to fungal infection possibly involving cytokinins (Hodges et al. 1968).

One problem with establishing a role for symbiotic fungi in SPB nitrogen nutrition is apparent when the SPB is compared to other bark beetles. *Ips calligraphus* and *I. grandicollis* have no mycangia, although they are consistently associated with the blue-stain fungus, *Ceratocystis ips*. The development of these beetles is not adversely affected by the absence of the blue-stain fungus or other microorganisms (Yearian 1967). Assuming that SPB and *Ips* beetles have similar nitrogen requirements, it is hard to postulate a role for SPB symbiotic fungi solely in terms of nitrogen nutrition.

Studies are needed of the nitrogen concentration of bark near galleries of SPB without symbiotic fungi. This would establish whether these fungi cause nitrogen redistribution or whether nitrogen accumulation is a passive process related to drying.

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Table 1. Nitrogen concentration of bark infested or not infested with SPB/microorganism complex.

	<u>Total nitrogen ($\mu\text{g}/\text{mg}$ dry wt.)</u>
Uninfested bark	4.3
Infested bark	6.9**

**Significantly different from uninfested bark based on a t-test ($P < 0.01$).